6 CARBOXYHEMOGLOBIN % IN BLOOD BY VISIBLE SPECTROMETRY

6.1 Summary

6.1.1 **Instrument option 1:** A dilute hemolysate of blood is treated with sodium dithionite to reduce oxyhemoglobin and/or methemoglobin; carboxyhemoglobin remains unaffected. The absorbance of this solution is scanned 650 nm to 500 nm and measured at 541 nm and 555 nm. The absorbance ratio of A_{541nm} / A_{555nm} is calculated and the percent carboxyhemoglobin is determined from the calibration curve.

6.1.2 **Instrument option 2:** Oxyhemoglobin (HbO_2) and carboxyhemoglobin (HbCO) are selectively differentiated based on the observation that if one component of a two-component system exhibits the same extinction at each of the two selected wavelengths, ΔA will be proportional to the concentration of the second component. The absorbance of HbO_2 is effectively cancelled by a double-wavelength measurement at 530 nm and 583 nm. ΔA reflects only HbCO concentration.

6.2 Specimen Requirements

6.2.1 Approximately 0.5 mL of whole blood

6.3 Reagents And Standards

6.3.1 Ammonium hydroxide

6.3.2 Sodium dithionite (sodium hydrosulfite)

6.3.3 Ammonium hydroxide (NH_4OH), 0.4%. Pipet 15.9 mL of concentrated NH_4OH into a 1L volumetric flask. QS to volume with dH_2O.

6.4 Calabrators, Controls, And Internal Standards

1.4.1 IL Test™Multi-4™ CO-Oximeter Controls, Instrumentation Laboratory Company, Lexington, MA.

6.5 Apparatus

6.5.1 1 cm UV-VIS cuvetttes.

6.5.2 **Instrument option 1:** Beckman DU® 7400 Spectrophotometer.

6.5.3 **Instrument option 2:** Shimadzu UV-160 Spectrophotometer.

6.6 Procedure

6.6.1 Add approximately one to three drops of case sample to a 1 cm cuvette containing 2-3 mL of 0.4% NH_4OH solution (try to achieve an absorbance maximum at approximately 1A) and mix by inversion. Case samples are run in duplicate.

6.6.2 Prepare a sample blank by adding approximately 2-3 mL of 0.4% NH_4OH solution to a blank cuvette

6.6.3 Negative and positive blood control samples are prepared as single samples

6.6.4 **Instrument option 1, Beckman DU® 7400 Spectrophotometer**

6.6.4.1 Scan blank

6.6.4.2 Scan the absorbance spectrum from 650 nm to 500 nm of each control sample and case sample.

6.6.4.3 Remove the cuvette after the wavelength scan is complete (an absorbance curve is displayed by the instrument). Add approximately 10 mg sodium dithionite to the cuvette (add the same amount to the duplicate cuvette if performing a case sample). Invert cuvettes to mix. Place one of the cuvettes into the spectrophotometer. The instrument will read a second scan within five minutes. After the reduced scan is read, place the second cuvette into the spectrophotometer and scan again. Print scans/results.

6.6.5 **Instrument option 2, Shimadzu UV-160 Spectrophotometer**

6.6.5.1 Scan blank

6.6.5.2 Scan sample from 700 nm to 450nm. If the absorbance is greater than 1.15, dilute sample with NH_4OH and scan again. Save scan in channel 1.

6.6.5.3 Measure absorbance of peak (570 nm). Calculate mg of total hemoglobin:

$$\text{Total hemoglobin} = \frac{\text{Abs @ 570nm x 10}}{9.3}$$

6.6.5.4 Measure the absorbance at 528 nm and 583 nm.

6.6.5.5 Remove sample cuvette and add a small amount of sodium dithionite to reduce oxyhemoglobin and methemoglobin. Mix by inverting the cuvette several times. Wait 5 minutes for the reaction to finish.

6.6.5.6 Scan again from 700 nm to 450 nm. Save second scan in channel 2. Print a copy of both scans in overlay mode.

6.6.5.7 Transfer data to worksheet.

6.7 Calculation

6.7.1 Using **Instrument option 1**: Calculate the ratio of the absorbance at 541nm / 555nm and determine the percent carboxyhemoglobin for the negative, positive control samples and the case samples from the calibration curve.

6.7.2 Using **Instrument option 2**: Calculate $\Delta Abs = Abs_{528nm} - Abs_{583nm}$. Convert this number to mg carboxyhemoglobin using the standard curve that plots known mg carboxyhemoglobin versus ΔAbs. Calculate the percent carboxyhemoglobin in the specimen using the equation $\%COHb = \frac{\text{mg carboxyhemoglobin}}{\text{Total Hemoglobin}} \times 100$.

6.7.3 The standard curve was obtained by saturating negative blood with known concentrations of HbCO and plotting the known %HbCO samples (10, 20, 50, 70 and 100% HbCO) versus ΔA. The curve is linear from 7-60% HbCO.

6.8 Quality Control and Reporting

6.8.1 Analyze at least one level of the controls with each group of case samples.

6.8.2 The LOQ for the assay is 7 % saturation and the ULOL is 60% saturation carboxyhemoglobin. Results below the LOQ are reported as "carbon monoxide less than 7% saturation". Results greater than the ULOL are reported as "carbon monoxide greater than 60% saturation".

6.8.3 Samples of questionable quality as may be reported as "unsuitable for analysis". A distinct minimum should appear in the unreduced hemoglobin spectrum and yield a ratio of A_{min}/A_{max} of about 0.8 or less. The absorbance minimum should be at least 20% lower than either adjacent maxima. A less distinct minimum may indicate that the sample is unsuitable for analysis (insufficient hemoglobin).

6.9 Notes

6.9.1 The exact concentration of hemoglobin in NH_4OH is not critical but the resulting absorbance maxima should be approximately 1 A. Ordinarily one to three drops of whole blood per 3 ml NH_4OH should be sufficient. More than four or five drops per 3 ml may indicate that the nature of the sample is questionable. If this occurs, the sample may be reported as "unsuitable for analysis".

6.10 References

6.10.1 Tietz, Norbert W., Ph.D. and Fiereck, A., M.S. Annals of Clinical Laboratory Science, Vol. 3, No. 1 pp. 36-42, 1973

6.10.2 B.L. Levine, Principles of Forensic Toxicology, American Association for Clinical Chemistry, Inc., pp. 330-337, 1999.

6.10.3 Ramieri, A., Jr., Jatlow, P. and Seligson, D. New method for rapid determination of carboxyhemoglobin by use of double-wavelength spectrophotometry (ΔA Method). Clinical Chemistry, Vol. 20, No. 2, 1974.

6.10.4 van Kampen, E.J .and Klouwen, N. Tidschr. Geneeskd, 98, pp. 161-164, 1954.